Comp. Biochem. Physiol., Vol. 60B, pp. 267 to 269
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0305-0491/78/0615-0267$02.00/0



# THE EFFECTS OF MAGNESIUM ON STATE 3 RESPIRATION OF LIVER MITOCHONDRIA FROM CONTROL AND COLD-ACCLIMATED RATS AND HAMSTERS

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(*Received* 19 *September* 1977)

**Abstract** 1. Increasing the $Mg^{2+}$ concentration results in a depression of succinoxidase-linked state 3 respiration of liver mitochondria from both control and cold-acclimated rats and hamsters.

2. It appears that in the cold-acclimated hamster, liver mitochondrial respiration is more sensitive to changes in $Mg^{2+}$ levels than that of the rat.

## INTRODUCTION

It is well known that in laboratory rodents cold acclimation results in an enhancement of liver mitochondrial state 3 respiration (respiration in the presence of added ADP) (Panagos *et al.*, 1958; Smith & Fairhurst, 1958; see also Masoro, 1976). The mechanisms by which this enhancement occurs have not been fully determined, but $Mg^{2+}$ has been implicated as one of the factors which can influence mitochondrial metabolism; for example, Bygrave (1967) and Wacker & Williams (1968) postulated that alterations in intracellular $Mg^{2+}$ may play a central role in the control of cellular respiration.

Panagos *et al.* (1958) and Smith & Fairhurst (1958) hypothesized that a decrease in coupling of oxidative phosphorylation in liver mitochondria of cold-acclimated rats was reponsible for the observed increase in mitochondrial succinate oxidation and, furthermore, they postulated that this uncoupling of oxidative phosphorylation is a necessary intracellular adjustment for survival in the cold.

McBurney & Radomski (1973) made a comparison of the effects of $Mg^{2+}$ on the oxidation of succinate in rat liver mitochondria from cold-acclimated and control rats. They found that as $Mg^{2+}$ levels are increased in the reaction mixture, liver mitochondrial state 3 respiration is depressed in both cold-acclimated and control rats. We wished to determine whether or not the decreased succinoxidase state 3 respiration seen in rat liver mitochondria with increased $Mg^{2+}$ (McBurney & Radomski, 1973) would also occur in mitochondria from a different rodent species the golden hamster (*Mesocricetus auratus*). Furthermore, we were interested in investigating this $Mg^{2+}$-induced metabolic suppression in hamster liver mitochondria, since these mitochondria show more marked changes in response to temperature acclimation than do those from rat liver (Chaffee *et al.*, 1961). We felt that if these inhibitory effects by $Mg^{2+}$ were greater in hamster liver mitochondria, then perhaps hamster liver mitochondria might afford an even more sensitive model system for future studies on $Mg^{2+}$ effects on mitochondria thermogenesis.

Our singular interest in studying state 3 respiration arose from the fact that it is considered to be representative of maximal mitochondrial respiratory capacity. Our use of succinate as substrate was for the purpose of comparing our results with those presented by McBurney & Radomski (1973).

## MATERIALS AND METHODS

Adult male Sprague–Dawley rats (350–450 g) and male hamsters (90–130 g, *Mesocricetus auratus*) were individually caged and fed Purina Laboratory Chow and water *ad libitum*. They were acclimated for 6–12 weeks at either 6 ± 1°C (cold) or 23 ± 1°C (control). Animals were sacrificed by decapitation. Livers were immediately removed, weighed and placed in ice-cold 0.25 M sucrose. Mitochondria were isolated according to the method of Johnson & Lardy (1967).

All succinoxidase assays were made at 37°C, using the reaction mixture and polarographic system of Estabrook (1967). Protein content was determined by the method of Lowry *et al.* (1951).

An analysis of variance was made on correlated data from the same fresh mitochondrial samples, and in all cases where a significant $F$ value was obtained, further analysis of differences between pairs of means was carried out by the Newman–Kuels method.

## RESULTS AND DISCUSSION

Table 1 shows the effects of $Mg^{2+}$ on rat liver mitochondrial state 3 respiration. In mitochondria from control and cold-acclimated rats 10 mM $Mg^{2+}$ caused a significant decrease in state 3 respiration as compared to respiration at 5.0 mM $Mg^{2+}$. Thus, the data confirms the findings of McBurney & Radomski (1973). However, we did not find any significant difference in the amount of inhibition between 10 and 15 mM $Mg^{2+}$, although the means seem to show a trend in that direction. Unlike McBurney & Radomski (1973) we made an additional study of succinoxidase with 2.5 mM $Mg^{2+}$. At that level, state 3 respiration of both control and cold-acclimated liver mitochondria was not significantly different from that at 5.0 mM $Mg^{2+}$ (Table 1).

Table 1. Magnesium effects on liver mitochondrial succinoxidase activity (state 3) of control and cold-acclimated rats

| $Mg^{2+}$ (mM) | Control $Q_{O_2}$* | P | Cold-acclimated $Q_{O_2}$* | P |
|---|---|---|---|---|
| 2.5 | 66.80 | - | 91.76 | — |
| 5.0 | 66.43 | NSD | 86.74 | NSD |
| 10.0 | 59.54 | 0.05 | 81.08 | 0.01 |
| 15.0 | 55.24 | 0.01 | 78.36 | 0.01 |
| 5.0 | 66.43 | | 86.74 | — |
| 10.0 | 59.54 | 0.01 | 81.08 | 0.05 |
| 15.0 | 55.24 | 0.01 | 78.36 | 0.05 |
| 10.0 | 59.54 | — | 81.08 | — |
| 15.0 | 55.24 | NSD | 78.36 | NSD |

* In $\mu$l $O_2$/mg protein per hr.

In all cases, cold-acclimated $Q_{O_2}$ values were significantly higher than those of control values ($P \leq 0.01$) at the same $Mg^{2+}$ concentrations.

Control and cold-acclimated hamster liver mitochondrial state 3 respiration rates are shown in Table 2.

In the control hamster 10 and 15 mM $Mg^{2+}$ produced an inhibition of state 3 respiration when compared to respiration with 2.5 mM $Mg^{2+}$, but 5 mM $Mg^{2+}$ did not. On the other hand, in making comparisons of state 3 respiration at 5, 10 and 15 mM $Mg^{2+}$ in control hamster liver mitochondria (Table 2), only 15 mM $Mg^{2+}$ caused a suppression when compared to 5 mM $Mg^{2+}$. In a comparison of state 3 respiration between 10 and 15 mM $Mg^{2+}$, there was no significant difference. Thus, control hamster liver mitochondria are not much different from those of the rat in their sensitivity to the metabolic suppression by $Mg^{2+}$.

In the case of the cold-acclimated hamster, however, we observed that succinoxidase state 3 respiration was most sensitive to $Mg^{2+}$ concentration changes of all liver mitochondria samples studied (see Table 2 and Fig. 1). As is seen in Table 2, there was a consistently significant decline in state 3 respiration

Table 2. Magnesium effects on liver mitochondrial succinoxidase activity (state 3) of control and cold-acclimated hamsters

| $Mg^{2+}$ (mM) | Control $Q_{O_2}$* | P | Cold-acclimated $Q_{O_2}$* | P |
|---|---|---|---|---|
| 2.5 | 55.60 | — | 81.15 | — |
| 5.0 | 54.06 | NSD | 75.62 | 0.01 |
| 10.0 | 48.84 | 0.05 | 69.65 | 0.01 |
| 15.0 | 45.94 | 0.01 | 65.09 | 0.01 |
| 5.0 | 54.06 | — | 75.62 | — |
| 10.0 | 48.84 | NSD | 69.65 | 0.01 |
| 15.0 | 45.94 | 0.05 | 65.09 | 0.01 |
| 10.0 | 48.84 | — | 69.65 | — |
| 15.0 | 45.94 | NSD | 65.09 | 0.05 |

* In $\mu$l $O_2$/mg protein per hr.

In all cases, cold-acclimated $Q_{O_2}$ values were significantly higher than those of control values ($P \leq 0.01$) at the same $Mg^{2+}$ concentrations.

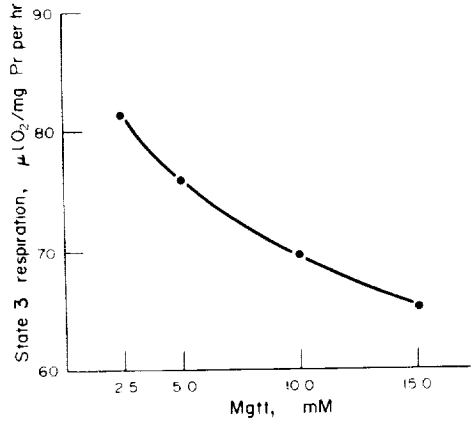


Fig. 1. $Mg^{2+}$ effects on succinoxidase state 3 respiration of cold-acclimated hamster liver mitochondria.

as the $Mg^{2+}$ levels were elevated. This is graphically illustrated in Fig. 1. Thus, for studies on the effects of $Mg^{2+}$ on mitochondrial metabolism, liver mitochondria from cold-acclimated hamsters seem to provide a very sensitive model. It is interesting to consider the fact that the hamster must become acclimated to the cold before it can hibernate. Whether or not $Mg^{2+}$ plays any significant physiological role in the induction of hibernation remains to be demonstrated by repeating similar studies involving other species of hibernators which are in a physiological state of preparedness for hibernation. In addition, in heat acclimation in the hamster, where there is extreme suppression of the activity of liver mitochondrial oxidative enzymes (Cassuto & Chaffee, 1966), the question of the effects of altered $Mg^{2+}$ levels warrant investigation.

*Acknowledgements*—This work was supported by ONR Grant NR 201066, NIH Grant AM 18895-01 and NASA-Ames NCA 1-OR 608-601.

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